Original article

BIOEQUIVALENCE STUDY OF THE BIOSIMILAR RECOMBINANT HUMAN ERYTHROPOIETIN ALPHA (RENOGEN®) AND EPREX® AFTER SUBCUTANEOUS ADMINISTRATION

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Abstract

To determine the bioequivalence of two formulations of recombinant human erythropoietin alpha (epoetin alpha 4000-IU), the two formulations; Renogen® and the Reference, Eprex® were administered to 18 healthy Thai male volunteers as a single subcutaneous dose according to a randomized two-way crossover design. Serial blood samples were collected over a period of 96 hours. The pharmacokinetic parameters were analyzed by noncompartmental analysis, and bioequivalence analysis (ANOVA) was carried out using logarithmically transformed data of the AUC, Cmax and untransformed Tmax. The elimination half-life of the test product (28.7 h) and the reference (31.1 h) were comparable. The median Tmax of the test product (12.0 h, range 4-15 h) was slightly slower than that of the reference (11.0 h, range 8-15 h). The ANOVA showed no statistically significant differences between the AUC and Cmax values or between the test and the reference preparations. The mean (90% CI) for the ratios Test/Reference for $\text{AUC}_{0-t}$, $\text{AUC}_{0-\infty}$ and Cmax were 1.03 (0.97-1.10), 1.01 (0.96-1.06) and 0.99 (0.90-1.08), respectively, within the bioequivalence range of 0.80-1.25. The study concluded that the test product is bioequivalent to the reference.


Keywords: bioequivalence, recombinant human erythropoietin alpha, Renogen®, Eprex®

Erythropoietin (EPO) is a glycoprotein growth factor that stimulates erythrocyte proliferation and differentiation. Endogenous EPO is produced in response to tissue hypoxia by the kidney. Once released, EPO distributes to the bone marrow, where it binds to a receptor on the surface of committed erythroid progenitors and is internalized to stimulate a rapid expansion of erythroid progenitors as well as inducing the release of reticulocytes. In normal subjects, the ranges of plasma EPO levels varied from 3.3-13.5 IU/L (mean 6.70 IU/L), 6-32 IU/L and 10-30 IU/L. However, changes in plasma EPO concentration are known to exhibit a circadian rhythm. EPO was found at its lowest mean level at 08:00 hours and had increased by 42% at 16:00 hours it
increased to the highest level by 60% at 20:00 hours.\(^{2}\) The recommended sampling time for EPO level is between 08:00-12:00 hours. The average EPO level in patients with chronic renal failure (creatinine clearance < 40 mL/min) is usually low, due to loss of functional tissues and cells responsible for EPO production.\(^{4}\) The anemia of chronic renal failure is due to inability of the kidney to produce adequate amounts of EPO as well as reduction in red cell survival. Nowadays, treatment of anemia from chronic renal failure has been revolutionized by the availability of recombinant human erythropoietin and renal failure patients will most likely respond to treatment with the exogenous EPO. Exogenous EPO for clinical use is produced by recombinant DNA technology and named recombinant human erythropoietin (rHuEPO) or epoetin. Epoetin alfa, epoetin beta, epoetin gamma, and epoetin omega are recombinant human erythropoietins derived from a cloned human erythropoietin gene.\(^{5}\) All have the same 165 amino acid sequence, but differ in the glycosylation pattern. Epoetin-alpha and epoetin-beta are available for clinical use in Thailand. The efficacy of epoetin may depend on the route of administration. Clinical studies have demonstrated that the subcutaneous (SC) route offers a few advantages over intravenous (IV) administration. For instance, SC administration is more convenient, as it does not require any venous access. When compared to the IV route, SC administration significantly prolongs the higher levels of serum erythropoietin, thus sustaining the stimulation of erythropoiesis.\(^{6}\) Although the bioavailability of SC administration is lower than IV, (bioavailability of 30-36% for epoetin alpha),\(^{4,7}\) a weekly dosage of epoetin required for therapeutic effect has been reported as 15-50% lower through the SC route when compared with IV administration.\(^{8}\) The dose-sparing effect has been attributed to the extended half-life after SC administration, resulting in a sustained stimulation of erythroid progenitor cells [half-lives for epoetin alpha after IV vs SC routes were 4-11 h vs 19-25.3 h].\(^{5}\) Furthermore, a sudden drop in circulating erythropoietin levels after IV administration may cause lysis of young erythrocytes. The adverse effects reported in renal failure patients included headache, hypertension, and seizures. The dose of rHuEPO must be adjusted to obtain a gradual rise in hematocrit level, over a 2- to 4-month period, until final hematocrit levels of 33% to 36% are reached. Subcutaneous administration may cause redness, swelling, or itching at the site of injection. Pure red cell aplasia (PRCA) associated with neutralizing antibodies to endogenous EPO has been reported in chronic renal failure patients with long-term use of rHuEPO.\(^{9}\) The majority of cases were observed after subcutaneous administration of epoetin-alpha, once human serum albumin had been removed from the formulation.\(^{10}\) Although the global incidence of this phenomenon is relatively low, rHuEPO should be discontinued and the patient should not be switched to another product, as anti-erythropoietin antibodies may cross-react with other epoetin.

Recently rHuEPO alpha has been developed and marketed in Thailand. Since subcutaneous administration of different preparations of rHuEPOs may result in different pharmacokinetic profiles, this study investigated the pharmacokinetics of the test product in comparison with Eprex\(^{8}\).
Objective
To compare the bioequivalence of the test product (Renogen®) and Eprex® after subcutaneous administration at an equal dose in healthy Thai male volunteers.

Materials and methods
Eighteen healthy Thai male volunteers aged between 19-29 years old (mean 22.89±3.18 years old and 23.00±1.87 years old for the T-R and R-T sequence, respectively) with a body mass index within 18.8–24.6 kg/m² (mean 20.30±1.18 years old and 21.46±1.79 years old for the T-R and R-T sequence, respectively) participated in this study. They were in good health on the basis of medical history, physical examination and routine blood test. Volunteers with known contraindication or hypersensitivity to the epoetin component were excluded as well as those with known hypersensitivity to human albumin products and mammalian cell-derived products. Volunteers with a known history of drug abuse, alcohol consumption and cigarette smoking were also excluded. The study was approved by the Research Ethics Committee of Chiang Mai University, Thailand and all volunteers signed the informed consent form prior to participation in the study.

Study drugs

Method of drug administration and blood sample collections
The study was a randomized, two-period, two-sequence, crossover study. On the study day, the volunteers received a single dose subcutaneous administration of either the test product or reference on the upper arm at 09:00 hours. The wash out period between each study visit was 21 days, after which the volunteers were crossed over to receive the alternative product. On each study day, a total of 12 blood samples (3 mL each) were collected at predose (0 hour) and 1, 2, 4, 8, 12, 15, 18, 24, 48, 72 and 96 h after dose administration. The plasma sample was separated promptly and immediately freeze at -70 °C until assay. Erythropoietin concentration in plasma was determined by the ELISA method using a Quantikine® IVD® EPO Kit (R&D System Inc. MN, USA).

Statistical and data analysis
Maximal plasma concentration (Cmax, mU/mL) and time to reach the peak concentration (Tmax, h) were obtained directly by visual inspection of each volunteer’s plasma concentration-time profile. The area under the plasma concentration-time curve (AUC) from time 0-infinity (AUC0-∞, mU.h/mL) and half-life (t1/2, h) were determined by non-compartmental analysis. The slope of the terminal log-linear portion of the concentration-time profile was determined by least-squares regression analysis and used as the elimination rate constant (Ke). The elimination half-life was calculated as 0.693/Ke. The AUC from zero to the last quantifiable
point \( (C_t) \) was calculated using the trapezoidal rule, and extrapolated AUC from \( C_t \) to infinity \( (\text{AUC}_{t-\infty}) \) was determined as \( C_t/K_e \). Total AUC\(_{\text{t-\infty}} \) was the sum of AUC0-t + AUC\(_{t-\infty} \). The calculation was performed by using the TopFit, pharmacokinetic data analysis program for PC.

An analysis of variance (ANOVA) was performed to determine the statistical differences of pharmacokinetic parameters (\( \text{AUC}_{0-\infty} \), \( C_{\text{max}} \), and \( T_{\text{max}} \)), which represented the extent and rate of drug absorption. Statistical analysis of AUC and \( C_{\text{max}} \) was performed on the logarithmically (ln) transformed data. The 90% confidence interval for the ratio of AUC as well as \( C_{\text{max}} \) values of the test preparation over those of the reference product were estimated using the following equation:

\[
90\%\ CI\ (\mu T-\mu R) = \left( \frac{X_t - X_R}{t\times S^2/n} \right) \pm t_0.1 \sqrt{\frac{S^2}{n}}
\]

- \( X_t \) and \( X_R \) are the observed mean of the (ln) transformed parameters (either \( C_{\text{max}} \) or AUC) for the test product (T) and the reference (R).
- \( S^2 \) is the error variance obtained from the ANOVA.
- \( n \) is the number of volunteers.
- \( t0.1 \) is the tabulated two-tail t value for 90% CI.
- \( v \) is the number of degrees of freedom of the error mean square.

The antilogarithm of the confidence interval \( (\mu T-\mu R) \) expressed the bioequivalence as a ratio of test and reference \( [\mu T/\mu R] \). The bioequivalence acceptance criteria required that the 90% CI for the ratio Test/Reference of the AUC\(_{0-\infty} \) and \( C_{\text{max}} \) fell within the interval of 0.8-1.25\(^{(10-11)} \).

Results and discussion

All volunteers completed the study without any serious adverse events. During the first visit, volunteer No. 14 received treatment (5 days of cephalexin at 250 mg qid, paracetamol at 500 mg) for fever and tonsillitis.

The mean baseline erythropoietin concentration (mU/mL) for the test [6.40±4.18 (range 3.79-21.99)] and reference [6.05±2.24 (range 2.77-13.37)] showed no statistically significant difference \( (p=0.1294) \) and was comparable to those values reported\(^{(1-3)} \). After subcutaneous administration, the erythropoietin plasma concentration increased slowly. The median \( T_{\text{max}} \) of the test (12.0 h, range 4-15 h) was slightly slower than that of the reference (11.0 h, range 8-15 h), and the mean (90% CI) for \( T_{\text{max}} \) differed (h) by 0.83 \([-0.59-2.26]\) (bioequivalence range ±2.06 h). The delay in absorption after the subcutaneous route resulted in a sustained erythropoietin concentration above baseline levels for 72 h in both preparations. The elimination of half-life, and the elimination

<table>
<thead>
<tr>
<th>Pharmacokinetic parameters</th>
<th>Test Product</th>
<th>Reference product</th>
<th>90% CI (BE range 0.80-1.25)</th>
</tr>
</thead>
<tbody>
<tr>
<td>( C_{\text{max}} ) (mU/mL)</td>
<td>74.08±17.00</td>
<td>75.35±19.66</td>
<td>0.99 (0.90-1.08)</td>
</tr>
<tr>
<td>AUD (mU/h/mL)</td>
<td>2706.52±381.60</td>
<td>2578.94±525.92</td>
<td>1.03 (0.97-1.10)</td>
</tr>
<tr>
<td>AUC (mU.h/mL)</td>
<td>3619.41±540.42</td>
<td>3075.01±571.01</td>
<td>1.01 (0.96-1.06)</td>
</tr>
<tr>
<td>( T_{\text{max}} ) (h)</td>
<td>11.1±3.12 (12.0)</td>
<td>10.3±2.27 (11.0)</td>
<td></td>
</tr>
<tr>
<td>( t_{1/2} ) (h)</td>
<td>28.7±4.88</td>
<td>31.1±10.79</td>
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</table>

PK parameters present as mean ± SD; \( T_{\text{max}} \) present as [Mean ± SD (median)]
rate constant of the test (28.69 h, 0.0244 h⁻¹) and reference (31.09 h, 0.0242 h⁻¹) were similar and comparable to those values reported [average t₁/₂ 20 h, range 6.94-48.27 h]. The mean plasma concentration-time curves of the test and reference product were also comparable (Fig 1). The relative bioavailability (Frel) calculated from Cmax, AUC₀₋ₜ and AUC₀₋∞ of Renogen®/Eprex® was 101%, 104% and 102%, respectively. From the ANOVA table, the intra-subject coefficient of variation (%CV) estimated from S² obtained from the ANOVA after logarithmic transformation, for the AUC₀₋ₜ, AUC₀₋∞ and Cmax, was 11%, 8% and 16%, respectively. According to the nomograms and tables of Diletti,[13] the power of test obtained from this study for AUC₀₋ₜ, AUC₀₋∞ and Cmax was more than 90% for the sample size of 18. The average Cmax (mU/mL) of Renogen® (74.08) and Eprex® (75.35) showed no significant difference, with a mean (90% CI) of the ratios of 0.99 (0.90-1.08). Similarly, the extent of absorption in both preparations was bioequivalent based on the mean (90% CI) of 1.03 (0.97-1.10) and 1.01 (0.96-1.06) for the ratios of the AUC₀₋ₜ and AUC₀₋∞, respectively. We concluded that Renogen® was bioequivalent to Eprex®, with respect to the rate and extent of absorption.

References
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การศึกษาชีวสมมูลของยา RECOMBINANT HUMAN ERYTHROPOIETIN ALPHA 2 ค่ารับเนื้อที่โดยการฉีดเข้าใต้ผิวหนัง

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บทที่ ที่ 1 การศึกษาชีวสมมูลของยา recombinant human erythropoietin alpha ขนาด 4000-IU 2 ซึ่งระหว่างยา Renogen® และยาต้นแบบ Eprex® ในอาสาสมัครชายไทยสุขภาพดี 18 ราย โดยอาสาสมัครจะได้รับยา 2 ครั้ง โดยการฉีดเข้าใต้ผิวหนังดังนี้ (ขั้นตอนการศึกษา
แบบสุ่มสลับชนิด 2 ทาง ทำการเก็บตัวอย่างเลือดเวลาที่กำหนดนาน 96 ชั่วโมง นาระดับยาในเลือดไปเล่มอย่างเท่ากันและผลลัพธ์ชีวสมมูลโดยวิธีทางสถิติผลการศึกษาพบว่าค่าชีวสมมูลของยาหลอด (28.7 ชั่วโมง) มีความใกล้เคียงกับยาต้นแบบ (31.1 ชั่วโมง) และเวลาที่ความเข้มข้นของยาสูงสุดในเวลาของยาหลอด (12 ชั่วโมง) จะขึ้นกว่ายาต้นแบบ (11 ชั่วโมง) แล้วน้อย แต่ความเข้มข้นของยาหลอดจะสูงกว่ายาต้นแบบที่ใช้ในการก่อนคิดชีวสมมูลของยาที่สูงไม่แตกต่างกัน โดยมีช่วงค่าความเชื่อมั่นที่ร้อยละ 90 ของอัตราส่วนของพื้นที่ใต้โค้งของกราฟความเข้มข้นของยาในเวลาที่สูงสุดที่ทำให้เกิดการเปลี่ยนแปลงของยาสูงสุดของยาที่ใช้ในการก่อนคิดชีวสมมูลของยาเท่ากับ (AUC0-t) และความเข้มข้นสูงสุดของยาในเวลาที่ยาวที่สุด (AUC0-∞) แต่ยังอยู่ในช่วงที่ 0.80-1.25 และค่าสูงสุดที่ อัตราส่วนของชีวสมมูลกันทั้งในเซ็ตปริมาณและอัตราส่วนของการรักษา

คำสำคัญ: ชีวสมมูล, Renogen®, Eprex®